09/831,419 C of C

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PATENT, TRADEMARK, COPYRIGHT AND RELATED INTELLECTUAL PROPERTY LAW

June 28, 2005

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: U.S. Patent No.: 6,806,260
Issued: October 19, 2004
Inventor: Yura Hirofumi et al.
Our Docket: 33550

Sir:

A Certificate of Correction under 35 U.S.C. 254 is hereby requested to correct Patent Office printing errors in the above-identified patent. Enclosed herewith is a proposed Certificate of Correction (Form No. PTO-1050) for consideration along with appropriate documentation supporting the request for correction.

It is requested that the Certificate of Correction be completed and mailed at an early date to the undersigned attorney of record. The proposed corrections are obvious ones and do not in any way change the sense of the application.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date indicated below.

Amanda Wittine	Amanda Wittine	June 28, 2005
Name of Depositor	Signature of Depositor	Date

We understand that a check is not required since the errors were on the part of the Patent and Trademark Office in printing the patent.

Very truly yours,

Paul A. Serbinowski, Reg. No. 34429

PAS:alw
Enclosures

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,806,260 PAGE 1 OF 2
DATED : October 19, 2004
INVENTOR(S) : Yura Hirofumi et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Column 7:

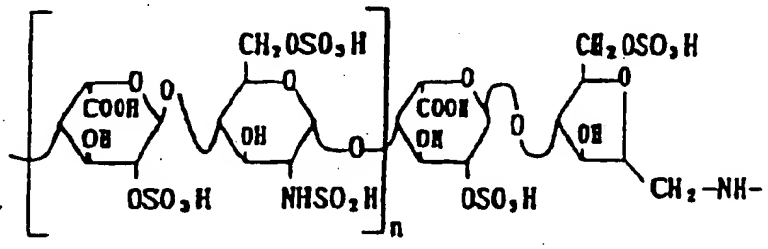
Line 56, please delete "degree of substitutions", and insert therefor --degree of substitution--.

In Column 10:

Line 23, please delete "$CH_2—(CH_2)_n—O—(CH_2CH_2O)_m—CH_2—CONH—$ (b)", and insert therefor -- $CH_3—(CH_2)_n—O—(CH_2CH_2O)_m—CH_2—CONH—$ (b)--

In Column 11:

Please delete the equations found on lines 1-28, and insert the following therefor:



(Derived from Heparin)

(Continued on page 2)

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PATENT NO. 6,806,260

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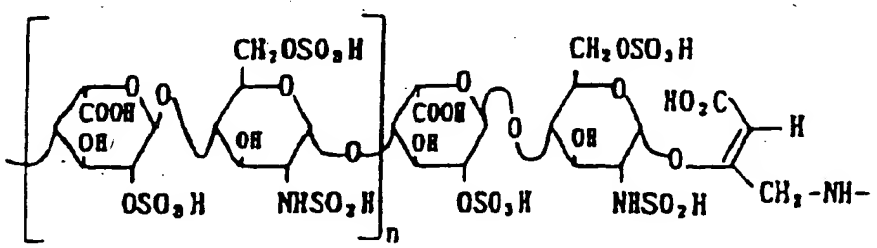
UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,806,260
DATED : October 19, 2004
INVENTOR(S) : Yura Hirofumi et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In Column 11:

(Continued from page 1)



(Derived from Heparin)

Line 57, please delete "issue", and insert therefor --tissue--.

In Column 14:

Line 31, please delete "30 degrees", and insert therefor --80 degrees--.

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PATENT NO. 6,806,260

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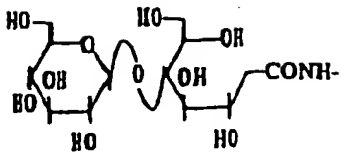
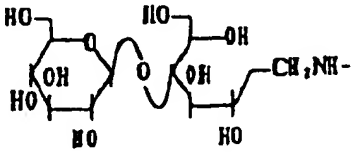
Sheets taken from original specification

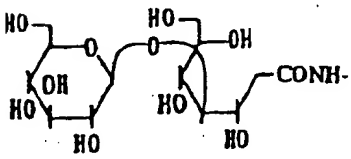
(vi) Derived from cellobiose



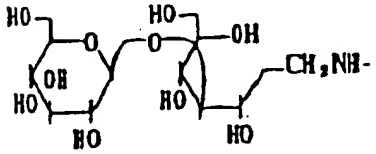
and



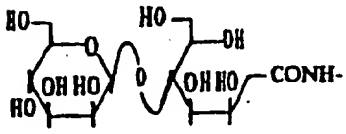
(v) Derived from laminaribiose



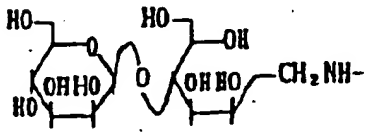
and



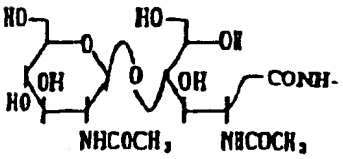
(vi) Derived from mannobiose



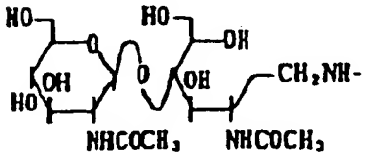
and



(vii) Derived from N-acetylchitobiose



and

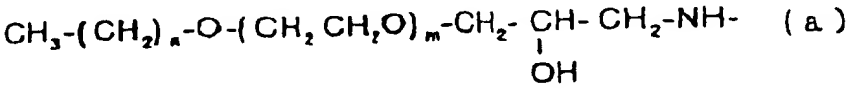


Of the carbohydrate side chains given in the above (i)-(vii), those on the left side represent residual groups incorporated by means of condensation between a carboxyl group on the carbohydrate and a 2-position amino group on the chitosan, while those on the right side represent residual groups bound by a Schiff base.

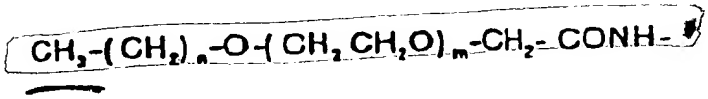
While the degree of substitution of 2-position amino groups in the glucosamin units of chitosan by carbohydrate side chains can be changed according to the physical properties desired in the final chitosan derivative, the degree of substitution should generally be in the range of 0.1-80%, preferably 0.5-60%, more preferably 1-40%. Here, the "degree of substitution" of the carbohydrate side chain is the level to which the amino groups in the 2-position of the carbohydrate units constituting the chitosans are substituted by carbohydrate side chains, and denote the proportion of substituted amino groups with respect to the total number of free amino groups and substituted amino groups at the 2-position of the carbohydrate units constituting the chitosans. In the present specification,

carboxyl group with the chitosan in the presence of a condensing agent, or a method of inducing a reaction between a polyoxyalkylene alkyl ether derivative having an acid chloride group with a hydroxyl group or amino group in the chitosan.

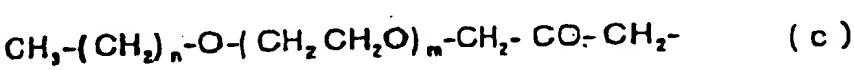
For example, when incorporating a polyoxyalkylene alkyl ether group with an epoxy group on its terminal into an amino group in the chitosan, the amphipathic group R2 in the above-given formula (4) is expressed by the following formula (a), and when incorporating a polyoxyalkylene alkyl ether group with an aldehyde group on its terminal into an amino group of the chitosan, the amphipathic group R2 of the formula (4) is expressed by the following formula (b). Additionally, when binding a polyoxyalkylene alkyl ether group with an acid chloride group on its terminal to the 3- or 6-position hydroxyl group of the chitosan, the amphipathic groups R2' or R2" in the above formulas (1)-(4) are expressed by the following formula (c). In the below formulas (a)-(c), n and m are repeating units numbering 1 or more.



(a)



(b)



(c)

The degree of incorporation of amphipathic groups in the chitosan derivatives of the present invention is not particularly restricted, but should be within the range normally of 5-70%, preferably 15-55% based on the change in weight of the chitosan derivative after incorporation.

Furthermore, in the present invention, it is preferable to further add to the chitosan derivative having incorporated therein a carbohydrate side chain and a photo-reactive functional group, as a fourth functionalization, a function of promoting healing which is an additional function heavily desired in wound dressing, by incorporation of glycosaminoglycans.

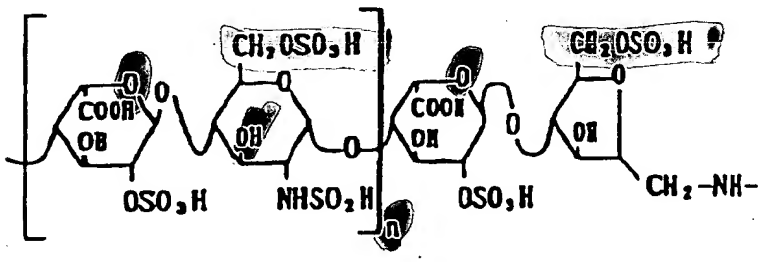
The healing promotion effect, in addition to wound repair, optimizes turnover of keratinocytes in the skin-care area, thus contributing to prevention of wrinkles.

While it has been suggested that chitosans naturally have a healing promotion effect, there have been reports that further healing promotion can be expected by ionic complexation of the glycosaminoglycans which are naturally-occurring acidic mucopolysaccharides with the basic groups of the chitosan (see Krats et al., Sc and J Plat Reconstr Hand Surg, 31, 119-123 (1997)). That is, the cell growth factors for stimulating the proliferation of fibroblasts and smooth muscle cells, which occurs during the healing process, are activated by binding to sulfated carbohydrates in the glycosaminoglycans.

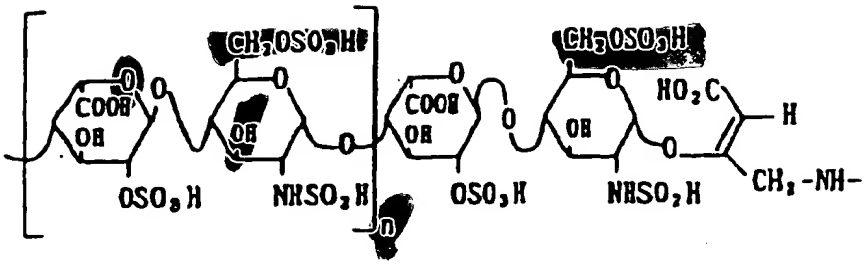
The incorporation of glycosaminoglycans to the chitosan of the present invention is not by the conventional ionic complexation method, and they are incorporated to the 2-position amino groups of the glucosamin unit of formula (1) by covalent bonds. The coupling method may, for example be the same method as the incorporation method for carbohydrates already explained, but in order to preserve sulfated carbohydrates to which cell growth factors can bind, it is possible to use a coupling method using aldehyde groups in which glycosaminoglycans are generated by means of periodic acid or nitrous acid decomposition.

Aside therefrom, coupling can be performed by binding through the above-mentioned chemical reaction to an insoluble self-crosslinked chitosan film due to photo-irradiation, or by binding by means of ionic complexation.

Specific examples of glycosaminoglycans incorporated in this way are those expressed by the following formulas, but there is no restriction to these.



(Derived from Heparin)



(Derived from Heparin)

The degree of substitution of the glycosaminoglycans in the chitosan derivatives of the present invention is not particularly restricted, but should normally be within the range of 1-40%, preferably 10-30%.

In the chitosan derivative of the present invention, at least one substituent group can be appropriately chosen for incorporation from among a carbohydrate having a reducing terminal (first function), a photo-reactive functional group (second function), an amphipathic group (third function) and a glycosaminoglycan (fourth function) according to the intended use.

For example, by providing both a photo-reactive functional group and an amphipathic group, it is possible to obtain a chitosan derivative, which forms a hydrogel having both greater strength and water retaining ability. A chitosan derivative such as this would be a novel functional material capable of forming a chitosan having a certain degree of wound healing, adhesion prevention, humectants and anti-bacterial effects into an insoluble gel with a desired strength in a short period of time, which could be widely applied in the field of health care such as in medicine and cosmetics. In particular, chitosan derivatives incorporating carbohydrates excel in solubility in the neutral region, so that they may be made into solutions in biological buffer solutions or cultures. Furthermore, chitosan derivatives having photo-reactive functional groups form thick aqueous solutions at a concentration of 0.1 wt% or more, and after application to tissue, can be made to form an insoluble gel which adheres firmly with the tissue within a few minutes by irradiation by ultraviolet rays of a predetermined intensity. As a result, it can be freely coated on or implanted in burns, tissue deficient areas, surgical openings, cavities generated by losing teeth, bone deficient portions or the like, then irradiated for a short period of time to

and Compound 1-B1 (0.5% maltose-substituted chitosan derivative) prepared in Example 1(1) were separately dissolved in 100 ml of a 50 mM aqueous TEMED solution. 0.35 g of EDC and 0.2 g of azidobenzoic acid were added to each chitosan derivative solution, and allowed to react for 72 hours. Unreacted substances of a molecular weight of 10,000 and below in the reaction solution were removed by ultrafiltration, to obtain chitosan derivatives incorporating carbohydrates and photo-reactive functional groups (first and second functionalizations) (hereinafter referred to respectively as "Compound 1-A1-a" and "Compound 1-B1-a"; degree of substitution by azidobenzoic acid, both 2.5 %).

(3) Preparation of Chitosan Derivative Incorporating Amphipathic groups (Third Functionalization)

1 g of a hydrochloride of the chitosan (Compound 1) used in Example 1 was dissolved in 40 ml of purified water, and 7.76 g of lauryl alcohol polyethylene glycol (15 repeating units) glycydyl ether (EX-171; Nagase Kasei Kogyo). After allowing to react for 24 hours at 80 degrees, methanol was added in excess to reprecipitate the chitosan ingredients. After dialysis, the result was freeze-dried to obtain a chitosan derivative incorporating an amphipathic group (hereinafter referred to as "Compound 1-I").

In the same manner as described above aside from using the compound 1-A1 (lactose-substituted chitosan derivative) prepared in Example 1(1) instead of the chitosan (Compound 1), a chitosan derivative with a carbohydrate and an amphipathic group (first and third functionalizations) was obtained (hereinafter referred to as "Compound 1-A1-I").

In the same manner as described above aside from using the compound 1-a (azidobenzoic acid-substituted chitosan derivative) prepared in Example 1(2) instead of the chitosan (Compound 1), a chitosan derivative with a photo-reactive functional group and an amphipathic group (second and third functionalizations) was obtained (hereinafter referred to as "Compound 1-a-I").

In the same manner as described above aside from using the compound 1-A1-a (lactose- and azidobenzoic acid-substituted chitosan derivative) prepared in Example 1(2)